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**Bioaccessibility of the bioactive peptide carnosine during *in vitro* digestion of cured beef meat**

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1   **ABSTRACT**

2   A bioactive compound is a food component that may have impact on health. Its bioaccessibility,  
3   defined as the fraction released from the food matrix into the gastrointestinal tract during digestion,  
4   depends on compound stability, interactions with other food components and supramolecular  
5   organization of food.

6   In this study, the effect of pH on the bioaccessibility of the bioactive dipeptide carnosine was  
7   evaluated in two commercial samples of the Italian cured beef meat Bresaola, at two key points of  
8   digestion: before the gastric and after the duodenal phases. The digestion process was simulated  
9   using an *in vitro* static system, while Capillary Zonal Electrophoresis and <sup>1</sup>H Nuclear Magnetic  
10   Resonance were used for quantitative analysis.

11   The gap between the total carnosine content, measured by CZE, and its free diffusible fraction  
12   observable by NMR spectroscopy, was 11% and 19% for two independent Bresaola products,  
13   where such percentages represent the fraction of carnosine not accessible for intestinal absorption  
14   because adsorbed to the food matrix dispersed in the digestion fluid.

15

16   **Keywords:** carnosine, Bresaola, *in vitro* digestion, bioaccessibility, bioactive compounds, <sup>1</sup>H-  
17   NMR, CZE.

18

## 19 INTRODUCTION

20 Bioactive compounds are extra nutritional constituents of food that typically occur in small  
21 quantities but can have a positive impact on body functions or conditions, and may ultimately  
22 influence health.<sup>1</sup> For their potential positive effects, they have been extensively studied in the last  
23 decade, isolated from different food sources and chemically and functionally characterised. Most of  
24 the research has focused on secondary plant materials such as phytosterols or polyphenols; meat and  
25 meat products, despite being recognised as good suppliers of high-grade proteins, vitamins and  
26 minerals, are generally not considered as sources of bioactive substances. Yet, research has made  
27 progress in the meat segment too, and today many compounds found in meat, i.e. small peptides,  
28 carnosine, anserine, taurine, creatinine, and coenzyme Q10 have been recognized as bioactives with  
29 a large spectrum of physiological actions.<sup>2-4</sup>

30 Carnosine is a  $\beta$ -alanine and L-histidine dipeptide, which is distributed widely and abundantly in  
31 excitable tissues (particularly muscle and nervous tissues) of several animal species. Although its  
32 physiological role has not been completely understood yet, many beneficial actions have been  
33 attributed to carnosine, such as being an antioxidant and a free-radical scavenger, an anti-glycating  
34 and an ion-chelating agent, and a wound healing promoter.<sup>5</sup> These biological activities may explain  
35 the ability of carnosine to exert an antiaging activity at cellular and whole animal levels<sup>6</sup> and to  
36 restrain some oxidative-based diseases such as diabetes,<sup>7-9</sup> atherosclerosis,<sup>10</sup> metabolic distress  
37 syndrome,<sup>11</sup> and also ischemia–reperfusion damage in different organs.<sup>12-14</sup>

38 After meat digestion the amount of dietary carnosine becoming available for intestinal absorption  
39 depends not only on its original concentration in food (related to animal species and tissue type),  
40 but also on its bioaccessibility, i.e. the fraction of the compound that is released from the food  
41 matrix in the gastrointestinal tract during digestion, and is available for intestinal absorption.<sup>15</sup>  
42 Nutrient bioaccessibility is affected by digestion conditions (pH, enzymes concentration and

43 activity), compound stability, interactions with other food components, as well as the  
44 supramolecular organization of the food.<sup>16</sup> Particularly, the pH of the environment is a critical  
45 parameter, being able to modify the net charge of a molecule, its structure and consequently the  
46 degree of interaction with the solvent and other food molecules.

47 In this work, the effect of pH on the solubility and in turn bioaccessibility of carnosine was assessed  
48 in Bresaola, a typical beef-based product of Northern Italy (Valtellina), which is produced by curing  
49 the intact beef muscle through different processing steps including a careful selection and skilful  
50 trimming cuts of meat, followed by a dry salting step, during which wine, spices and sugar (with the  
51 aim of encouraging microbial phenomena responsible for much of the aging of the product) are  
52 added, then the meat is stuffed into natural or artificial casings and sent to the next stage of drying  
53 in special cells for a slow maturation. As this substrate is consumed crude, the evaluation of  
54 carnosine content is not expected to be affected by alterations due to the cooking process, which can  
55 cause a reduction of carnosine up to 50% in other beef products, depending on the cooking  
56 procedure.<sup>17,18</sup> The digestion of Bresaola occurring *in vivo* in the human gastro-intestinal tract was  
57 simulated using an *in vitro* static system,<sup>19</sup> and the soluble free compound in the digestion fluid was  
58 evaluated by coupling <sup>1</sup>H Nuclear Magnetic Resonance spectroscopy (<sup>1</sup>H-NMR) and Capillary  
59 Zone Electrophoresis (CZE). These techniques were operated at different pH values to obtain a  
60 global description of the free fraction of the molecule at three key points of digestion, i.e. the end of  
61 the oral phase, after 180 and 300 min of duodenal digestion.

62 To the best of the Authors knowledge this research work represents the first study estimating the  
63 effect of pH on the interactions of carnosine with the digested meat matrix, and therefore on its  
64 bioaccessibility. The presence of emulsifiers, metal ions, fats, biopolymers and other chemical  
65 species able to bind the bioactive compounds could sequestrate them, thus limiting their free  
66 diffusion through the extracting digestion fluid, from food matrix, where bioactive molecules are

67 embedded, to the gut mucosa where their absorption occurs. The modification of bioaccessibility  
68 due to interactions with the food matrix is a key factor that should be considered while evaluating  
69 the potential significance of bioactives in human health. Each compound, indeed, is susceptible of  
70 chemical exchange between environments, being either freely diffusible in the digestion fluids or  
71 being adsorbed on the surfaces of the macromolecular food matrix; this could affect the bioactive  
72 bioaccessibility, and therefore effectiveness, when it is delivered in the human diet as part of a  
73 complex food matrix and not as a discrete molecule.

74

## 75 MATERIALS AND METHODS

76 All chemicals and solvents were of the highest analytical grade, and were purchased from Sigma–  
77 Aldrich (St. Louis, MO), except where indicated. The two types of commercial Bresaola, named B1  
78 and B2, were bought in a local market.

79

### 80 Cured meat sample preparation for carnosine quantification in raw material.

81 Bresaola meat samples were prepared for carnosine quantification by grinding them with a common  
82 mincer. Five grams of minced meat where then homogenized with 10 mL of distilled water for 4  
83 min at 25000 rpm using a homogenizer. Therefore, the pH was adjusted to 2.5 with 37% HCl and,  
84 after centrifugation for 30 min at 11500 rpm at 4 °C, the supernatant was filtered through a 0.45 µm  
85 filter, boiled for 10 min, centrifuged for 20 min at 11500 rpm, 4 °C and filtered again using a 0.22  
86 µm filter. For CZE analysis the filtered sample was diluted 1:20 with 100 mM phosphate buffer pH  
87 2.5 (BioRad Laboratories, California). For  $^1\text{H}$ -NMR spectroscopy 160 µL of deuterium oxide  
88 ( $\text{D}_2\text{O}$ ), containing 10 mM 3-TrimethylSilyl-Propanoic-2,2,3,3-d4 acid sodium salt (TSP), as  
89 chemical shift calibration standard, were added to 1 mL of filtered sample, and the pH was adjusted  
90 to the investigated value by adding 10% NaOH or 1 M HCl.

91

92 **Cured meat sample preparation for carnosine pH dependence evaluation**

93 The pH dependence of carnosine in Bresaola meat was analysed by  $^1\text{H}$ -NMR in samples prepared  
94 as described in the previous section. In these experiments, before the first centrifugation, the pH  
95 was adjusted, by adding 10% NaOH or 1 M HCl, to the following values: 2, 2.5, 3.5, 5, 6, 7 and 8.

96 Afterward, the samples were centrifuged at 14000 rpm for 5 min in order to remove precipitates.

97

98 **Cured meat *in vitro* digestion**

99 The Bresaola samples were digested in triplicate according to Bordoni,<sup>19</sup> following the scheme  
100 reported in Figure 1.

101 In details, human digestion was simulated *in vitro* inside a 100 mL flask, kept at 37 °C in a water  
102 bath on a magnetic stirrer equipped with a heating plate.<sup>20</sup> Chemical composition of the digestive  
103 fluid, pH and residence periods were adjusted to mimic the physiological conditions. Three samples  
104 were collected during digestion: P1, before the gastric phase, when the food enters the stomach and  
105 the pH decreases to 2); P2 and P3 after 180 and 300 min from the beginning of the duodenal phase,  
106 respectively.

107 Samples P2 and P3 were acidified to pH 2 with 37% HCl to stop hydrolysis by the pancreatic  
108 enzymes. P1, P2 and P3 samples were stored at -80 °C before CZE and  $^1\text{H}$ -NMR experiments,  
109 before any further treatment.

110 For CZE, 500  $\mu\text{L}$  of samples P1-P3 were boiled for 10 min, immediately cooled and then  
111 centrifuged for 30 min at 9,500 X g and 4 °C; the supernatant was filtered through a 0.22  $\mu\text{m}$  before  
112 analysis.

113 Samples P1-P3 were prepared for  $^1\text{H}$ -NMR by adding 160  $\mu\text{L}$  of  $\text{D}_2\text{O}$ , containing 10 mM TSP, as  
114 chemical shift calibration standard, to 1 mL of each sample. After adjusting the pH to 7, the samples  
115 were centrifuged at 14000 rpm for 5 min in order to remove precipitates

116 **Carnosine quantification by CZE**

117 CZE analysis and data processing were performed on a Biofocus 2000 from BioRad (BioRad  
118 Laboratories, California). Uncoated fused-silica capillaries with a diameter of 50  $\mu\text{m}$  and effective  
119 length of 51 cm, were used for the analysis. The temperature during the analysis was maintained  
120 constant at 38 °C and samples were analyzed in a 110 mM phosphate buffer at pH 2.5 (BioRad  
121 Laboratories, California). The voltage was kept at 15 KV and the separated components were  
122 detected at 200 nm (UV), without derivatization. The quantification was performed in duplicate by  
123 area integration.

124

125  **$^1\text{H}$  NMR evaluation of carnosine concentration and carnosine pH dependence**

126 All  $^1\text{H}$ -NMR spectra were recorded at 25 °C on a Bruker US+ Avance III spectrometer operating at  
127 600 MHz, equipped with a BBI-z probe and a B-ACS 60 sampler for automation (Bruker BioSpin,  
128 Karlsruhe, Germany). Spectra were collected with a 90° pulse of 14  $\mu\text{sec}$  with a power of 10 W, a  
129 relaxation delay of 5 sec and an acquisition time of 2.28 s.

130 Spectra were registered by means of the first increment of a NOESY sequence, designed to suppress  
131 the HOD residual signal, while giving, for each proton, signals proportional to the concentration of  
132 the substance they belong.

133

134 **Statistical analysis**

135 Data normality and homogeneity of the variances were assessed by means of Shapiro-Wilks and  
136 Barlett tests respectively. Sample and pH effects on carnosine concentration were investigated

137 through ANOVA test, followed by *post-hoc* LSD test. Every calculation was performed by means  
138 of the correspondent packages implemented in R computational language. A trim value of 0.05 was  
139 considered, unless differently stated.

140

## 141 RESULTS AND DISCUSSION

### 142 Carnosine quantification in raw Bresaola meat by CZE and $^1\text{H-NMR}$ spectroscopy

143 In undigested raw samples, carnosine content was measured by both CZE and  $^1\text{H-NMR}$ , using the  
144 purified compound as reference standard. Measurements were performed at pH 2.5, which  
145 optimizes carnosine resolution through CZE.<sup>21</sup>

146 By CZE the content of carnosine was estimated at 628 mg/100 g and 671 mg/100 g in raw B1 and  
147 B2, respectively.

148 A typical  $^1\text{H-NMR}$  spectrum of bresaola extract, with carnosine highlighted, is reported in Figure 2.  
149 For the quantification of this molecule the signals at 7.30 and 8.60 ppm, corresponding to imidazole  
150 hydrogens Ha and Hb of the histidine residue, were considered, because they are singlets (thus  
151 granting the highest signal to noise ratio), and well resolved from signals ascribable to other  
152 molecules. In all the registered spectra it was possible to observe a 1:2 ratio between the area of  
153 these signals and those from  $\beta$ -alanine appearing in the range 2.65 - 2.80 ppm, corresponding to the  
154 two protons Hf and Hg, indicating that the amount of free histidine in all the samples was  
155 negligible.

156 By  $^1\text{H-NMR}$  the amount of measured carnosine was estimated 623.3 and 664.1 mg/100 g of raw  
157 products, in B1 and B2 samples respectively. The very low difference in carnosine concentration  
158 detected by  $^1\text{H-NMR}$  and CZE values ( $\Delta = 0.7\%$  and 1.0% for B1 and B2 samples, respectively)  
159 clearly indicates a good agreement between the two techniques, when working at the same acidic  
160 pH.

161 These values are higher than those reported in the literature for beef meat, whose carnosine content  
162 ranges from 32.6 mg/100 g in heart to 452 mg/100 g in semitendinosus muscle,<sup>22,23</sup> being generally  
163 higher in anaerobic, glycolitic, white muscle than in red, aerobic muscle.<sup>6</sup> It is conceivable that the  
164 higher carnosine content is related to the dehydration process that raw meat undergoes during  
165 Bresaola production, which increases the protein content from 20.5 to 32 g/100g.<sup>24</sup>

166

167 **Effect of pH on carnosine quantification in Bresaola meat**

168 Previous investigations have shown that the solubility of low molecular weight molecules during  
169 food digestion depends on the physiological pH of the different digestion environments, which can  
170 be modulated by the interaction with the food matrix undergoing digestion.<sup>25</sup> This especially holds  
171 for carnosine, whose net charge strongly depends on pH for the presence of three ionizable groups,  
172 namely the histidine  $\alpha$  carbossilic group ( $pK_a = 2.64$ ), the histidine imidazolic group ( $pK_a = 6.83$ )  
173 and the alanine  $\beta$  amminic group ( $pK_a = 9.51$ ).

174 To better elucidate the relationship between carnosine bioaccessibility and pH, the dipeptide  
175 concentration in raw B1 and B2 samples was quantified by  $^1\text{H-NMR}$  in the 2-8 pH range. As  
176 reported in Figure 3, in both samples the amount of free carnosine was maximum at pH below 2.5  
177 or above 8, and was characterized by a curvilinear relationship with pH, with a minimum around pH  
178 5.3, corresponding to the isoelectric point of meat proteins. In detail, carnosine concentration in  
179 solution appeared significantly different between pH 2 and 7 ( $P < 0.05$ ).

180 The quantification of carnosine alone in water solution did not show any change of concentration  
181 depending on pH (data not reported), pointing out that such behavior was peculiarly ascribable to  
182 the interaction between carnosine and the food matrix. The lowest recovery around meat proteins  
183 isoelectric point suggested in particular the interaction of carnosine with the hydrophobic patches  
184 over the surface of meat proteins.

185 Meat is characterized by its own pH, depending on the quality and manufacturing processes in case  
186 of curing, ageing or other technological transformations. The purpose of this study was to describe  
187 the effect of the digested meat matrix on the solubility of carnosine, which modulate its  
188 accessibility, e.g., the diffusion of the dipeptide towards the gut mucosa where the absorption will  
189 occur. The possible implication of this phenomenon on the kinetics of absorption should be taken  
190 into account when developing new meat products, by considering that matrix could be modified,  
191 thus reacting to digestion in different ways.

192

### 193 Carnosine bioaccessibility during *in vitro* Bresaola digestion

194 In order to study carnosine bioaccessibility during digestion, its concentration was measured in the  
195 soluble fraction of samples collected at different phases of an *in vitro* static digestion process. CZE  
196 and  $^1\text{H}$ -NMR were used in parallel at pH 2.5 and 7 respectively. pH values were adjusted before  
197 sample injection (CZE) or transfer to the NMR tube, respectively. As mentioned above,  
198 measurement at pH 2.5 optimizes carnosine resolution through CZE;<sup>21</sup> furthermore, it allowed a  
199 direct comparison between data obtained on raw samples and digestates. Measurement by  $^1\text{H}$ -NMR  
200 were performed at pH 7 to focus on the carnosine concentration at pH value similar to those found  
201 in the intestine, where absorption takes place.

202 Carnosine concentration in the soluble fractions of digestate obtained at three different time points  
203 (P1, P2, and P3), as the result of three repetitions of *in vitro* digestion processes of B1 and B2  
204 products, are reported in Table 1.

205 Carnosine concentration at P1 corresponds to the amount of compound brought into solution after  
206 the oral step, during which the meat is chewed in the presence of saliva. CZE analysis allowed  
207 comparing carnosine content at P1 and in raw, undigested samples, evidencing that in both samples

208 the simple action of chewing and subsequent acidification actually released the most of the  
209 dipeptide (96% and 90% in B1 and B2, respectively).

210 In both samples carnosine concentration measured by  $^1\text{H-NMR}$  at pH 7 was lower than by CZE, in  
211 agreement with the above reported data on the effect of pH on carnosine quantification.

212 Comparison was made between the gastric samples analyzed by CZE at pH 2.5 and the same  
213 samples analyzed by NMR after bringing the pH at 7. This change of pH may parallelize the jump  
214 occurring when the bolus exit the stomach and enter the duodenum.

215 As well, carnosine concentration at P2 and P3 (after 180 min and 300 min from the beginning of the  
216 duodenal digestion, respectively) was higher by CZE than by  $^1\text{H-NMR}$ . It is important to consider  
217 that the pH of the intestinal environment is close to 7, so  $^1\text{H-NMR}$  analysis actually mirrors the  
218 amount of carnosine available for absorption (i.e. the bioaccessible carnosine), values obtained with  
219 CZE being an artefact due to the condition of analysis.  $^1\text{H-NMR}$  showed that, after 180 min of  
220 duodenal digestion, the amount of compound found as the bioaccessible soluble fraction was 89%  
221 and 81% of the total extracted from the raw samples B1 and B2, respectively. Prolongation of the *in*  
222 *vitro* duodenal digestion for further 120 min (P3) did not lead to any significant increase in  
223 carnosine concentration, indicating that carnosine release from the food matrix is almost completed  
224 after 3 h of duodenal digestion.

225 Summarizing, in the present work two different techniques, CZE and NMR spectroscopy, were used  
226 in parallel to evaluate the carnosine concentration in two samples of the typical Italian cured meat  
227 based product Bresaola. Evaluation was performed on both the raw material and after *in vitro*  
228 digestion of samples. Both analysis were performed on raw Bresaola at pH 2.5, and results  
229 evidenced a good agreement between the two techniques. In digested samples, CZE analysis was  
230 still performed at pH 2.5, which represents the optimum pH value for the analysis, while NMR  
231 spectroscopy was performed at a pH value resembling the one in the intestine (pH 7). In this way, it

232 was possible to evidence the influence exerted by the matrix on the carnosine transfer from the food  
233 to the soluble fraction, avoiding interference due to the herein evidenced effect of pH on carnosine  
234 quantification.

235 The nutritional evaluation of a food as source of specific nutrient/s is usually based on the chemical  
236 composition of the food itself. This approach does not take into account the digestion process,  
237 which regulates the amount of nutrients that can be absorbed, leading to a handy approximation  
238 often exceeding the real role of the food in the satisfaction of the human metabolic needs. Nutrients  
239 availability depends on the food matrix in which they are embedded, and on food processing, that  
240 can interfere with the digestion process. An exhaustive evaluation of the nutritional meaning of a  
241 food should take into account, among others, the modifications occurring during the digestion  
242 process, since only nutrients that are absorbed have the possibility to modulate the physiological  
243 processes occurring in the human body.

244 Although the present study aimed at investigating the concentration of free carnosine in the gastric  
245 and duodenal digestion fluids, the same phenomenon may occur in meat samples before digestion.  
246 It is interesting to note that pH 5,3 is typical of meat, and at this pH carnosine has the lowest  
247 fraction as free diffusible molecule and this could prevent depletion of such dipeptide during drip  
248 loss.

249 The potentiality of NMR spectroscopy to describe the interactions between a bioactive compound  
250 and the food matrix, directly in the digestion fluid and at the physiological pH, provides a tool that  
251 may help differentiating meat products on the basis of their matrix effect. Matrix is difficult to be  
252 studied during digestion, but its definition may be inferred through the study of its chemical  
253 interactions with soluble molecules. The simple quantification of the total amount of each  
254 compound extracted from the meat prevent the differentiation of meat products on the  
255 supramolecular scale, i.e. on the basis of its matrix. NMR spectroscopy offers the opportunity to

256 leave the molecules in their physiological environments, including those characterizing the  
257 digestion process and for which the food matrix may play an important role for the diffusibility of  
258 the bioactive molecules from food to the gut mucosa.

259 An omic approach is needed to describe the food system and its relationship with the human  
260 system. This approach, known as foodomics, has gained momentum in recent years since it allows  
261 reaching a deeper level of knowledge about the nutritional value of a food, and of the relationship  
262 between food and health.

263

## 264 ABBREVIATIONS

265 **NMR**: Nuclear Magnetic Resonance; **CZE**: Capillary Zone Electrophoresis; **NOESY**: Nuclear  
266 Overhauser Effect SpectroscopY

267

## 268 REFERENCES

- 269 1. Kitts, D. D.; Weiler, K., Bioactive Proteins and Peptides from Food Sources. Applications of  
270 Bioprocesses used in Isolation and Recovery. *Curr. Pharm. Design* **2003**, *9*, 1309-1323.
- 271 2. Schmid, A., Bioactive substances in meat and meat products. *Fleischwirtschaft Int.* **2009**,  
272 89, 83-90.
- 273 3. Szterk, A.; Roszko, M., Simultaneous determination of free amino acids, l-carnosine, purine,  
274 pyrimidine, and nucleosides in meat by liquid chromatography/single quadrupole mass  
275 spectrometry. *J. Liq. Chrom. Rel. Technol.* **2013**, *37*, 664-680.
- 276 4. Saiga, A; Kanda, K.; Wei, Z.; Okumura, T.; Kaneko, T.; Nishimura, T., Hypotensive activity  
277 of muscle protein and gluten hydrolysates obtained by protease treatment, *J. Food Biochem.*, **2002**,  
278 26, 391-401.

- 279 5. Bellia, F.; Vecchio, G.; Cuzzocrea, S.; Calabrese, V.; Rizzarelli, E., Neuroprotective  
280 features of carnosine in oxidative driven diseases. *Mol. Aspects. Med.* **2011**, *32*, 258-266.
- 281 6. Hipkiss, A. R., Carnosine and Its Possible Roles in Nutrition and Health. In *Advances in*  
282 *Food and Nutrition Research*, Steve, L. T., Ed. Academic Press: 2009; Vol. 57, pp 87-154.
- 283 7. Riedl, E.; Pfister, F.; Braunagel, M.; Brinkkötter, P.; Sternik, P.; Deinzer, M.; Bakker, S. J.  
284 L.; Henning, R. H.; van den Born, J.; Krämer, B. K.; Navis, G.; Hammes, H. P.; Yard, B.; Koeppel,  
285 H., Carnosine Prevents Apoptosis of Glomerular Cells and Podocyte Loss in STZ Diabetic Rats.  
286 *Cell. Physiol. Biochem.* **2011**, *28*, 279-288.
- 287 8. Lee, Y.-t.; Hsu, C.-c.; Lin, M.-h.; Liu, K.-s.; Yin, M.-c., Histidine and carnosine delay  
288 diabetic deterioration in mice and protect human low density lipoprotein against oxidation and  
289 glycation. *Eur. J. Pharmacol.* **2005**, *513*, 145-150.
- 290 9. Pfister, F.; Riedl, E.; Wang, Q.; vom Hagen, F.; Deinzer, M.; Harmsen, M. C.; Molema, G.;  
291 Yard, B.; Feng, Y.; Hammes, H. P., Oral Carnosine Supplementation Prevents Vascular Damage in  
292 Experimental Diabetic Retinopathy. *Cell. Physiol. Biochem.* **2011**, *28*, 125-136.
- 293 10. Rashid, I.; van Reyk, D. M.; Davies, M. J., Carnosine and its constituents inhibit glycation  
294 of low-density lipoproteins that promotes foam cell formation in vitro. *FEBS Lett.* **2007**, *581*, 1067-  
295 1070.
- 296 11. Aldini, G.; Orioli, M.; Rossoni, G.; Savi, F.; Braidotti, P.; Vistoli, G.; Yeum, K.-J.;  
297 Negrisoli, G.; Carini, M., The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal  
298 function in Zucker obese rats. *J. Cell. Mol. Med.* **2011**, *15*, 1339-1354.
- 299 12. Pekcetin, C.; Kiray, M.; Ergur, B.; Tugyan, K.; Bagriyanik, H.; Erbil, G.; Baykara, B.;  
300 Camsari, U., Carnosine attenuates oxidative stress and apoptosis in transient cerebral ischemia in  
301 rats. *Acta Biol. Hung.* **2009**, *60*, 137-148.

- 302 13. Dobrota, D.; Fedorova, T.; Stvolinsky, S.; Babusikova, E.; Likavcanova, K.; Drgova, A.;  
303 Strapkova, A.; Boldyrev, A., Carnosine Protects the Brain of Rats and Mongolian Gerbils against  
304 Ischemic Injury: After-Stroke-Effect. *Neurochem. Res.* **2005**, *30*, 1283-1288.
- 305 14. Lee, J. W.; Miyawaki, H.; Bobst, E. V.; Hester, J. D.; Ashraf, M.; Bobst, A. M., Improved  
306 Functional Recovery of Ischemic Rat Hearts due to Singlet Oxygen Scavengers Histidine and  
307 Carnosine. *J. Mol. Cell. Cardiol.* **1999**, *31*, 113-121.
- 308 15. Fernández-García, E.; Carvajal-Lérida, I.; Pérez-Gálvez, A., In vitro bioaccessibility  
309 assessment as a prediction tool of nutritional efficiency. *Nutr. Res.* **2009**, *29*, 751-760.
- 310 16. Bordoni, A.; Laghi, L.; Babini, E.; Di Nunzio, M.; Picone, G.; Ciampa, A.; Valli, V.;  
311 Danesi, F.; Capozzi, F., The foodomics approach for the evaluation of protein bioaccessibility in  
312 processed meat upon in vitro digestion. *Electrophoresis* **2014**, *35*, 1607-1614.
- 313 17. Purchas, R. W.; Busboom, J. R.; Wilkinson, B. H. P., Changes in the forms of iron and in  
314 concentrations of taurine, carnosine, coenzyme Q<sub>10</sub>, and creatine in beef longissimus muscle with  
315 cooking and simulated stomach and duodenal digestion. *Meat Sci.* **2006**, *74*, 443-449.
- 316 18. Peiretti, P. G.; Medana, C.; Visentin, S.; Dal Bello, F.; Meineri, G., Effect of cooking  
317 method on carnosine and its homologues, pentosidine and thiobarbituric acid-reactive substance  
318 contents in beef and turkey meat. *Food Chem.* **2012**, *132*, 80-85.
- 319 19. Bordoni, A.; Picone, G.; Babini, E.; Vignali, M.; Danesi, F.; Valli, V.; Di Nunzio, M.;  
320 Laghi, L.; Capozzi, F., NMR comparison of in vitro digestion of Parmigiano Reggiano cheese aged  
321 15 and 30 months. *Magn. Reson. Chem.* **2011**, *49*, S61-S70.
- 322 20. Szterk, A., Chemical state of heterocyclic aromatic amines in grilled beef: Evaluation by in  
323 vitro digestion model and comparison of alkaline hydrolysis and organic solvent for extraction.  
324 *Food Chem. Toxicol.* **2013**, *62*, 653-660.

- 325 21. Žunić, G.; Spasić, S., Capillary electrophoresis method optimized with a factorial design for  
326 the determination of glutathione and amino acid status using human capillary blood. *J. Chromatogr.*  
327 *B* **2008**, *873*, 70-76.
- 328 22. Purchas, R. W.; Rutherford, S. M.; Pearce, P. D.; Vather, R.; Wilkinson, B. H. P.,  
329 Concentrations in beef and lamb of taurine, carnosine, coenzyme Q10, and creatine. *Meat Sci.* **2004**,  
330 *66*, 629-637.
- 331 23. Cornet, M.; Bousset, J., Free amino acids and dipeptides in porcine muscles: differences  
332 between 'red' and 'white' muscles. *Meat Sci.* **1999**, *51*, 215-219.
- 333 24. INRA Tabella di composizione di alimenti. <http://www.inran.it/>
- 334 25. Laghi, L.; Babini, E.; Bordoni, A.; Ciampa, A.; Danesi, F.; Nunzio, M. D.; Picone, G.; Valli,  
335 V.; Capozzi, F., Time Domain Measurements and High Resolution Spectroscopy are Powerful  
336 Nuclear Magnetic Resonance Approaches Suitable to Evaluate the In Vitro Digestion of Protein-  
337 rich Food Products. In *Magnetic Resonance in Food Science: Food for Thought*, van Duynhoven,  
338 J.; Van As, H.; Belton, P. S.; Webb, G., Eds. RSC Publishing: 2013; pp 201-212.

339

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**FIGURE CAPTIONS**

**Figure 1** Flow chart of human *in vitro* digestion. P1, P2 and P3 are the labels of samples collected at the beginning of the gastric phase (P1), and after 180 (P2) and 300 min (P3) from the beginning of the duodenal phase.

**Figure 2**  $^1\text{H}$ -NMR Spectrum of A) bresaola B1 sample at P1 digestion point (pH= 7.0); B) carnosine standard, showing the signals from L-histidine (Ha, Hb, Hc, Hd e He) and  $\beta$ -alanine (Hh, Hi, Hf, Hg) residues.

**Figure 3** Carnosine concentration as assessed by  $^1\text{H}$ -NMR in presence of meat samples B1 and B2 at different pH values. The error bars indicate the standard deviation calculated on 3 replicates.

**Table 1:** Carnosine Concentration of Bresaola B1 and B2, Measured by CZE and  $^1\text{H-NMR}$ , at the Beginning of the Gastric Digestion (P1) and After 180 and 300 min from the Beginning of the Duodenal Phase (P2 and P3). The Values of Three Replicates of Digestion and Their Average  $\pm$  Standard Deviation are Presented, Expressed in mg/100 g of Meat.

B1	CZE (pH 2.5) mg/100 g	$^1\text{H NMR}$ (pH 7) mg/100 g		$\Delta$ CZE - $^1\text{H-NMR}$
P1	620.3	600.73 $\pm$ 19.70	514.7	10.50%
	601		574.66	
	580.9		542.09	
P2	638.94	654.43 $\pm$ 13.85	519.57	17.32%
	658.76		584.29	
	665.6		569.58	
P3	608.89	631.78 $\pm$ 20.64	524.7	13.67%
	637.5		580.85	
	648.96		561.86	

B2	CZE (pH 2.5) mg/100 g	$^1\text{H NMR}$ (pH 7) mg/100 g		$\Delta$ CZE - $^1\text{H-NMR}$
P1	595.32	607.18 $\pm$ 17.44	519.3	17.21%
	599.02		513.01	
	627.2		521.74	
P2	712.726	659.57 $\pm$ 63.92	519.57	21.60%
	588.64		584.29	
	677.34		569.58	
P3	681.68	640.74 $\pm$ 57.41	524.7	15.32%
	575.12		580.85	
	665.42		561.86	

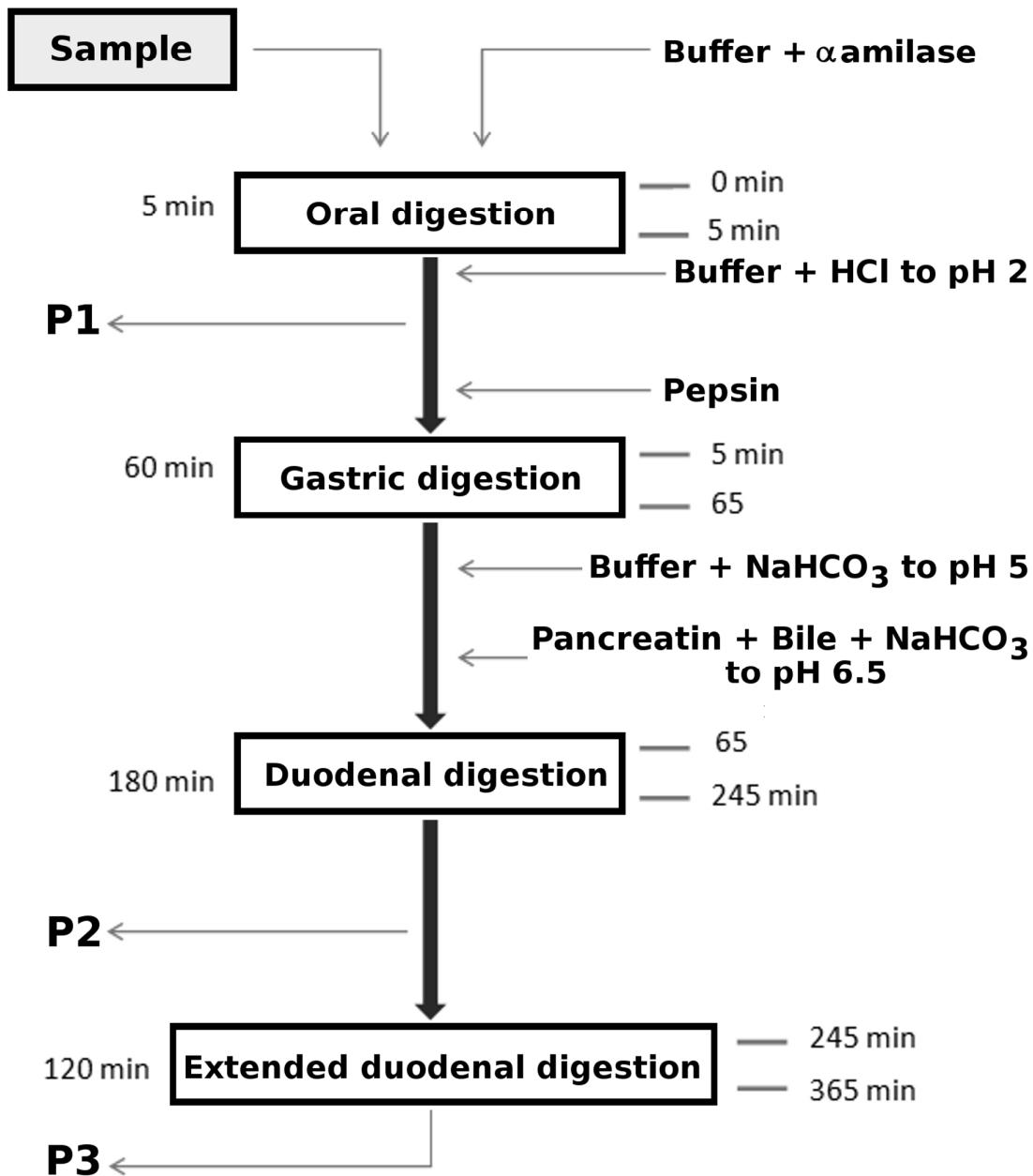


Figure 1

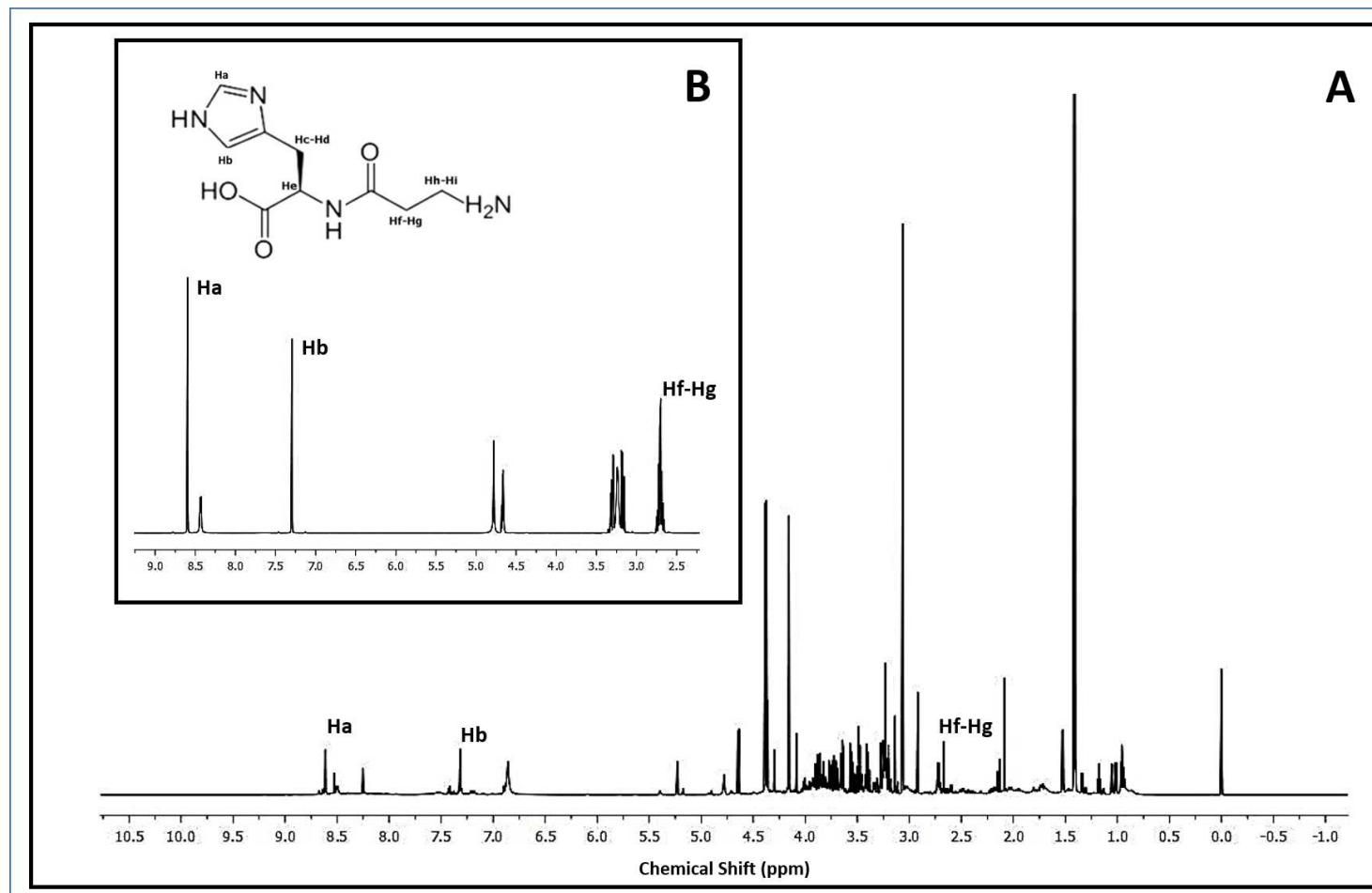


Figure 2

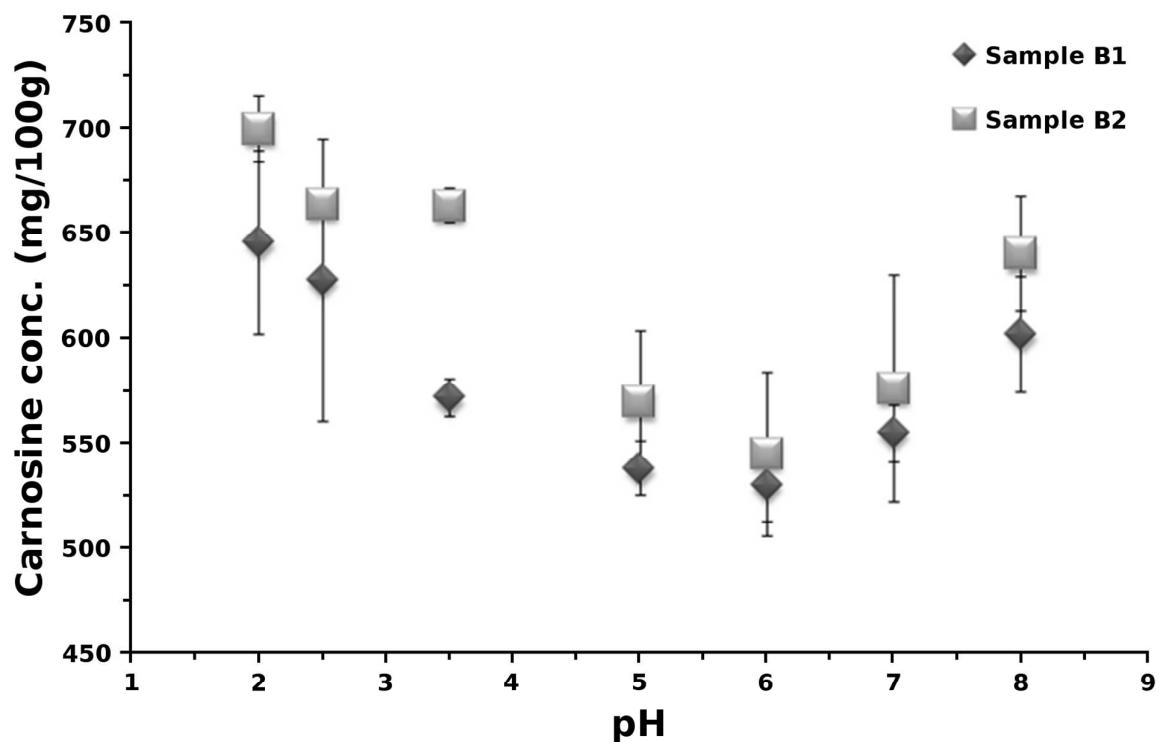


Figure 3

## Table of Contents Graphic (TOC)

